REMARKS

Reconsideration of the present application is respectfully requested in view of the above amendments and the following remarks. Claims 1, 15-17, 44, and 45 are currently pending and under examination. By the present Amendment, claims 15 and 16 are canceled, and claim 1 is amended to more particularly point out and distinctly claim certain embodiments of the invention. No new matter has been added by these amendments. Support for the amendments can be found in the specification as originally filed; for example, on page 6, lines 25-28; page 8, lines 12-13; and in the original claims. It should be noted that the above amendments are not to be construed as acquiescence with regard to the Examiner's rejections and are made without prejudice to prosecution of any subject matter removed or modified by this amendment in a related divisional, continuation or continuation-in-part.

Rejections Under 35 U.S.C. § 103

A. The Examiner rejects claim 1 under 35 U.S.C. § 103(a) as being allegedly obvious over Holtzman (U.S. Pat. No. 5,959,123) in view of Schatz (U.S. Pat. No. 5,932,433). The Examiner asserts that Holtzman teaches a biochip comprising a biotinylated receptor specifically immobilized on the chip through the receptor's biotinylation sequence motif, wherein the receptor is capable of binding a ligand, but agrees with the Applicants that Holtzman fails to teach biotinylation of the receptor protein carried out within a bacterial host. The Examiner then asserts that Schatz teaches biotinylation of a recombinantly expressed receptor protein within a bacterial host, and further asserts that it would have been obvious to include the *in vivo* biotinylation as taught by Schatz in making the biochip of Holtzman.

B. The Examiner rejects dependent claims 15 and 16 under 35 U.S.C. § 103(a) as being allegedly obvious over Holtzman, in view of Schatz, and in further in view of Tall *et al.* (U.S. Pat. No. 6,756,228). The Examiner relies on Holtzman and Schatz as discussed above, and further asserts that Tall *et al.* teaches a LOX-1 receptor immobilized to a substrate in order to detect the presence of LOX-1 activity. The Examiner asserts that it would have been obvious to combine the LOX-1 protein described in Tall *et al.* with the biochip described in

Holtzman and the *in vivo* protein biotinylation described in Schatz in arriving at the instant claims.

C. The Examiner rejects dependent claims 17 and 44 under 35 U.S.C. § 103(a) as being allegedly obvious over Brigham-Burke *et al.* (U.S. Pat. No. 5,395,587), in view of Holtzman, and in further view of Schatz. The Examiner relies on Holtzman and Schatz as discussed above, and further asserts that Brigham-Burke *et al.* teach a protein immobilized on a sensor chip substrate that conforms to a shape of an insertion site of a plasmon resonance device, allegedly rendering the instant claims obvious.

D. The Examiner rejects dependent claims 17 and 45 under 35 U.S.C. § 103(a) as being allegedly obvious over Muramatsu (*Analytical Chemistry*, 1987;59:2760-63), in view of Holtzman, and in further view of Schatz. The Examiner relies on Holtzman and Schatz as discussed above, and further asserts that Muramatsu teaches a protein immobilized on a crystal oscillator, which allegedly renders the instant claims obvious.

Applicants traverse these grounds for rejection and submit that the presently claimed subject matter satisfies the requirements of non-obviousness under 35 U.S.C. § 103(a). More specifically, Applicants submit that the Examiner has not established a *prima facie* case of obviousness with respect to the presently claimed subject matter. (*See In re Mayne*, 104 F.3d 1339 (Fed. Cir. 1997)) (UPSTO has the burden of showing a *prima facie* case of obviousness).

Applicants submit that the Examiner must at a minimum show that the combined references teach or suggest all the claim features, and even assuming, *arguendo,* that the combination of references teaches each claim feature, the Examiner must provide an explicit, apparent reason to combine these features in the fashion claimed by the Applicant. *See KSR v. Teleflex, Inc.,* No 04-1350 at 4, 14 (U.S. Apr. 30, 2007) ("A patent composed of several elements is not proved obvious merely by demonstrating that each element was, independently, known in the prior art"). The Examiner must also show that a person skilled in the art would have had a reasonable expectation of success in arriving at the claimed subject matter. M.P.E.P. § 2143.02 (*citing In re Merck & Co., Inc.*, 800 F.2d 1091 (Fed. Cir. 1986)). In the instant case, the cited references, alone or in combination, fail to teach or suggest each and every claim feature, and

also provide no apparent reason to combine their teachings with a reasonable expectation of success.

As an initial matter, Applicants note that claims 15 and 16 have been canceled, rendering moot the Examiner's rejection to these claims. Without acquiescence to any rejection, the subject matter of claims 15 and 16 has been incorporated by amendment into claim 1. Applicants respectfully submit that this amendment obviates the rejections outlined in sections A, C, and D above, since none of these combinations of references teach each element of the claimed receptor chip. Specifically, none of the cited references in A, C, or D teach or suggest a receptor chip wherein the receptor protein is LOX-1.

With respect to the rejection outlined in section B above, Applicants submit that the cited references, alone or in combination, fail to render the claimed receptor chip obvious. Applicants strongly emphasize that the Examiner must consider the claim as a whole when making a determination under 35 U.S.C. § 103. "In determining the differences between the prior art and the claims, the question under 35 U.S.C. §103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious." M.P.E.P. §2141.02, *citing Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983). In this light, Holtzman, Schatz, and Tall *et al.*, alone or in any combination, clearly fail to render the present invention obvious. Not only do they fail to teach each and every claim feature, but they also provide no apparent reason to combine their teachings with a reasonable expectation of success in achieving the presently claimed invention as a whole.

Specifically, Applicants submit that none of Holtzman, Schatz, or Tall *et al.* teach or suggest an *in vivo* biotinylated LOX-1 receptor protein obtained from bacterial inclusion bodies, wherein the LOX-1 receptor protein is re-folded and maintains its ligand binding functional activity. As conceded by the Examiner, Holtzman does not teach *in vivo* biotinylation of a receptor protein within a bacterial host, let alone the *in vivo* biotinylation of LOX-1 specifically.

Schatz fails to remedy the deficiencies in Holtzman. In particular, Schatz does not teach or in any way suggest the unexpected result that *in vivo* biotinylated LOX-1 can be

obtained from inclusion bodies in a bacterial host *and* correctly re-folded in a particular orientation on solid substrate, such that LOX-1 is capable of ligand binding. At best, Schatz teaches the expression and purification of maltose-binding protein (MPB) in *E. coli*, but nothing in Schatz even remotely describes using a LOX-1 receptor protein in an *in vivo* biotinylation protocol.

Tall *et al.* fail to remedy the noted deficiencies of both Holtzman and Schatz. Tall *et al.* merely describe the nucleotide sequence of various LOX-1 genes and their association with atherosclerosis, and at best prophetically mention using bacterially derived LOX-1 protein immobilized on a solid surface. But Tall *et al.* nowhere teach or in any way suggest using an *in vivo* biotinylated LOX-1, let alone an *in vivo* biotinylated, properly re-folded, ligand-binding LOX-1 obtained from inclusion bodies in a bacterial host, as recited in the instant claims. Thus, Holtzman, Schatz, and Tall *et al.*, alone or in combination, fail to teach each feature of claim 1 as amended and, therefore, fail to render obvious the presently claimed subject matter.

Even assuming, *arguendo*, that the cited references taught each feature of claim 1, Applicants submit that the presently claimed invention would not be obvious over this combination of references, since there is no apparent reason to combine their teachings with a reasonable expectation of success in arriving at the presently claimed receptor chip comprising a LOX-1 receptor protein. By way of general explanation, a person skilled in art of bacterial polypeptide production understands that receptor proteins are distinct from other proteins in general, and understands also that this distinction is especially notable with regard to post-translationally modified proteins, such as LOX-1. Since post-translation modification does not occur in bacterial hosts, the successful production of functional mammalian receptor protein, *e.g.*, LOX-1, in bacteria could not be expected. Indeed, the ability to do so demonstrates an unexpected advantage of the presently claimed subject matter.

More specifically, as noted herein, Holtzman fails to teach an *in vivo* biotinylated LOX-1 receptor protein produced in bacteria. Schatz does not remedy this deficiency, and further provides no apparent reason to use the *in vivo* biotinylation method described therein for producing *in vivo* biotinylated LOX-1. Rather, Schatz fails to provide any motivation or reasonable expectation of success in arriving at the presently claimed subject matter, as Schatz

does not teach a person skilled in the art how to overcome the well-known difficulties in expressing and purifying *in vivo* biotinylated, correctly re-folded, ligand-binding LOX-1 from a bacterial host.

As further explanation, Applicants submit that it is understood in the art that post-translational modification is often crucial for mammalian receptor proteins, such as LOX1, which in particular is known to be heavily post-translationally modified with sugar chains (*see, e.g.,* Kataoka *et al.*, *Journ. Biol. Chem.*, 2000;275(9):6573-6579 and Shi *et al.*, *Journ. Cell Sci.*, 2001;114:1273-1282, enclosed herewith). According to the understanding in the art, post-translational modification plays a key role in LOX-1 function, affecting both cell surface expression and ligand binding. *Id.* A person of ordinary skill in the art would, therefore, not reasonably expect *in vivo* biotinylated LOX-1 produced in a bacterial host to be capable of ligand binding, since LOX-1 produced by this method would not have been modified with the necessary sugar chains. Schatz does nothing to alter this expectation, as Schatz merely teaches the *in vivo* biotinylation of MBP, which is naturally produced in *E. coli*, and, therefore, requires no post-translational modification to maintain functionality. In view of these deficiencies in the prior art, Applicants submit that the combination of: (i) producing *in vivo* biotinylated LOX-1 protein obtained from inclusion bodies; and (ii) refolding the LOX-1 in a particular orientation on a solid substrate, such that the LOX-1 receptor protein has the ability to be specifically bound by its ligand, clearly establishes the non-obviousness of the presently claimed receptor chips.

Contrary to the Examiner's assertion (*see* the Action, page 5), Schatz also fails to show at all whether the *in vivo* biotinylated MBP described therein maintains its original function. In fact, Schatz merely tests MBP protein for biotinylation efficiency (*see, e.g.,* Example 17 at column 17, line 11), and does not demonstrate specifically whether *in vivo* biotinylated MBP can still bind maltose, its natural ligand. As previously made of record, Schatz also describes a limited intended use for *in vivo* biotinylated proteins, which use does not depend on preserving protein function (*see, e.g.,* column 14, lines 10-16). In direct contrast, the present application demonstrates empirically that *in vivo* biotinylated LOX-1 obtained from inclusion bodies in a bacterial host is correctly re-folded and capable of binding a ligand (*see, e.g.,* Figures 1-3, described on page 14, line 13 through page 15, line 2 of the specification). Schatz utterly

fails to teach or in any way suggest to a person of ordinary skill in the art that it is possible to preserve the ligand binding characteristics of *in vivo* biotinylated LOX-1 receptor protein obtained from inclusion bodies in a bacterial host, as recited in the instant claims.

As previously made of record and not fully appreciated by the Examiner, the deficiencies in both Holtzman and Schatz are especially acute given the understanding in the art at the time of filing, which appreciates the traditional difficulties associated with expressing and purifying post-translationally modified mammalian receptor proteins, such as LOX-1, in high amounts using a bacterial host (*see, e.g.,* page 3, lines 8-26 of the specification). These references do not even remotely suggest that LOX-1 receptor protein can be successfully adapted to *in vivo* biotinylation and expression protocols. Producing functional, re-folded proteins that can be identically oriented due to *in vivo* biotinylation in an amount sufficient to produce receptor chips was not previously attainable prior to the disclosure of the present application. A person of ordinary skill in the relevant art would, therefore, have no reasonable expectation of success in arriving at the claimed subject matter from reading either Holtzman or Schatz, as these references fail to teach or in any way suggest producing a ligand binding, correctly re-folded, *in vivo* biotinylated LOX-1 receptor protein from inclusion bodies in a bacterial host.

Tall *et al.* also fail in every respect to teach or suggest a way around the noted difficulties associated with expressing and purifying heavily post-translationally modified mammalian receptor proteins in high amounts using a bacterial host. Without specific guidance in this regard, mere mention of mammalian LOX-1 immobilized on a solid surface can not render obvious the presently claimed subject matter. As evidenced by the present application and the secondary references submitted herewith, a person of ordinary skill in the art at the time of filing would not have reasonably expected biotinylated, mammalian LOX-1 produced from inclusion bodies in bacteria to correctly re-fold and bind its ligand. Instead, Applicants' substantial inventive efforts unexpectedly succeeded in adapting *in vivo* biotinylation and expression protocols for use with a highly post-translationally modified mammalian receptor protein, allowing LOX-1 to be produced for functional solid phase immobilization at levels higher than previously found in the art. Applicants therefore submit that the presently claimed receptor chip is non-obvious in view of Holtzman, Schatz, and Tall *et al.*

With regard to the rejections described in sections C and D above, and in light of the failure of Holtzman, Schatz, and Tall *et al.*, alone or in combination, to teach each feature of independent claim 1 as amended, Applicants submit that the present amendments and above remarks also overcome the rejections of dependent claims 17, 44, and 45. In particular, neither Brigham-Burke *et al.* nor Muramatsu remedy the deficiencies as noted herein, as these references concededly fail to teach a biotinylated protein (*see* the Action, page 4) and, in particular, fail to teach an *in vivo* biotinylated LOX-1 that is capable of binding its ligand.

In view of the Remarks and Amendments provided herein, Applicants submit that claim 1 and those claims dependent therefrom satisfy the requirements of non-obviousness under 35 U.S.C. § 103, and respectfully request reconsideration and withdrawal of the Examiner's rejection.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Applicants respectfully submit that all the claims in the application are allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

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